TSS CHEMICAL TRANSFORMATION

Why to do this :

1. Insert plsmids notably into NM522 strains, or any other E.Coli strain.
2. It has been found more efficient than the CaCl2 transformation protocol, thus we recommend using it.

What you need :

1. Culture media : LB

- 10 g bactotrypton
- 5 g yeast extract
- 5 g NaCl
- 0,5 mL NaOH 10N
- Qsp 1 L

2. Antibiotics concentrations

Chloramphenicol (Cm) : 2 mg/mL
Tetracycline (Tet) : 1 mg/mL
Kanamycin (Kann) : 5 mg/mL
Ampicillin (Amp) : 10 mg/mL

→ 50 µL antibiotic / 5mL medium

3. Material :

- 100µL of TSS
- 1µL of DNA
- 900µL LB medium
- Ice
- Heating bath (42°C)
- 5mL of NM522 cell culture

How to do :

1. Cell culture

a) Monitor the OD600 of a 5mL culture of NM522 cells (or the strain you want to transform) in LB medium at 37°C. Proceed to the next step when it reaches 0.3-0.4 (not higher than 0.6), which takes approximately 3 hours.

b) Take 1 mL from the previous culture and centrifuge at 8000 rpm for 2mn

2. Transformation

a) Resuspend into 100µL of TSS. Starting from this step, keep the bacteria on ice.
b) Incubate on ice for 5-10mn.
c) Add 1µL of the DNA to be transformed
d) Incubate on ice for 10mn at least
e) Heat shock the bacteria by heating them at 42°C for 50s
f) Incubate on ice for 2mn
g) Add 900µL LB and mix by inverting the tube
h) Incubate for 1h at 37°C
i) Plate 100µL on LB medium with the appropriate antibiotic